REMARKS

Summary

On July 23, 2009, Applicant filed a Reply to the final Office Action dated March 23, 2009. In an Advisory Action mailed on August 20, 2009, the Examiner refused to enter the amendment. Applicant is now filing a Request for Continuing Examination, including the present Reply to the Office Action of March 23, 2009.

In the present Reply, applicant first re-assserts that it would not have been obvious for a skilled worker to combine a reference which allegedly discloses that certain statins can both inhibit HMG-CoA reductase and enhance osteoblastic differentiation of mammalian mesenchymal cells (MSCs) with a reference which discloses that some oxysterols can inhibit HMG CoA reductase, to arrive at the present claims, which are directed to a method for using oxysterols to enhance osteoblastic differentiation of MSCs. In brief: Just because some compounds which inhibit HMG-CoA reductase (such as certain statins) had been reported to enhance osteoblastic differentiation of MSCs, a skilled worker would not have expected, with a reasonable expectation of success, that other agents which inhibit HMG-CoA reductase (such as the oxysterols of the present invention) would also enhance osteoblastic differentiation. Stem cell differentiation is a complex and poorly understood biological phenomenon; and causality between stem cell osteoblastic differentiation and HMG-CoA reductase inhibition had not been previously demonstrated. Therefore, it would have been unpredictable at the time of filing of the present application whether an inhibitor of HMG-CoA reductase would, or would not, enhance osteoblastic differentiation of MSCs. The finding that oxysterols can, indeed, stimulate osteoblastic differentiation of MSCs was thus unexpected.

Furthermore, applicant now presents a publication (Parhami *et al.* (2002) *J. Bone & Mineral Res* 17, 1997-2003), which published contemporaneously with the filing of the present application, in which he and his colleagues reported that at least one statin inhibits HMG-CoA reductase but, contrary to the Examiner's allegation that agents which inhibit HMG-CoA reductase would also be expected to stimulate osteoblastic differentiation, this statin has the opposite effect, and *inhibits* osteoblastic differentiation of MSCs. This finding supports applicant's argument that there is not a causal relationship between these two phenomena, and, in fact, teaches away from there being such causality.

To support this argument, attached is a Declaration under 37 CFR 1.132 executed by the inventor, Professor Farhad Parhami, attesting that at the time the application was filed, neither he nor other researchers in the stem cell field would have been able to predict whether an agent that inhibits HMG-CoA reductase (such as an oxysterol) would, or would not, stimulate osteoblastic differentiation of MSCs.

The claims

Claims 1-3, 6-8, 11-12, 15-17, 19-21, 23-26, and 28 are pending. Claims 4-5, 9-10, 18, 22, 27, and 29-41 are withdrawn. Claims 13-14 are canceled. Some of the claims are amended to clarify the Markush claim language. The amendments do not add new matter, and do not narrow the scope of the claims.

Applicant requests that, once the claims currently under consideration are allowed, the claims reciting species which were not elected for preliminary examination (*e.g.*, claims 4, 5, 9 10, 18, 22 and 27) be examined. It is noted that claims 4, 5, 9 10, 18, 22 and 27 recite additional elements that were not recited in the examined claims. That is, they recite "further comprising" treating the mammalian mesenchymal cells with at least one secondary agent. Therefore, provided that the elected claims are deemed patentable, these additional claims, which merely add additional elements or steps, will not require a further search and examination, and should also be deemed to be patentable.

Obviousness rejections

The Examiner cites the Paralkar patent application (**"Paralkar"**) for its alleged disclosure of *in vitro* and *in vivo* methods for enhancing bone formation in mammals, including humans, comprising administering HMG-CoA reductase inhibitors such as statins in combination with prostaglandin agonists. The Examiner points in particular to disclosures in the reference that statins allegedly enhance the expression of mRNA for BMP-2, an agent that has been reported to induce osteoblastic differentiation.

Many of the statements that the Examiner attributes to Paralkar were, in fact, the characterization by Paralkar of other publications that are referred to in that patent application. Without discussing the statements in Paralkar in detail, applicant notes that the reference reports that certain statins allegedly enhance the expression of markers associated with

bone formation, or stimulate bone formation, presumably via a mechanism of stimulating the formation of certain BMPs (*e.g.*, BMP-2). However, the reference does not suggest or disclose that oxysterols can enhance osteoblastic differentiation.

The Examiner argues that because both oxysterols and statins have been reported to inhibit HMG-CoA reductase activity, it would have been obvious for a skilled worker to substitute oxysterols for the statins used by Paralkar, in order to induce osteoblatic differentiation (in MSCs), with a reasonable expectation of success.

However, there is a logical disconnect to the allegation that oxysterols could substitute for the statins: Paralkar did not demonstrate a causal relationship between the ability of statins to inhibit HMG-CoA reductase activity and their alleged ability to induce osteoblastic differentiation. Thus, even if it were the case that certain statins, which can inhibit HMG-CoA reductase, could induce osteoblastic differentiation in MSCs, this does not necessarily mean that OTHER agents which inhibit HMG-CoA reductase, such as oxysterols, would also induce osteoblastic differentiation in MSCs.

Biological phenomena, such as the physiology of osteoblastic differentiation, are complex and poorly understood. It would have been unpredictable at the time of filing the present application whether two different inhibitors of HMG-CoA reductase would have the same effect on, *e.g.*, osteoblastic differentiation. Absent the establishment of a causal link between the ability of an agent to inhibit HMG-CoA reductase and the ability of the agent to induce osteoblastic differentiation, there would have been no motivation for a skilled worker to substitute an oxysterol for the statins of the Paralkar reference, with the requisite reasonable expectation of success.

In fact, importantly, the inventor and his colleagues reported contemporaneously with the filing of the present application that at least one statin - metavastin (which is one of the statins listed by Paralkar, in paragraph [0011], line 9) - inhibits HMG-CoA reductase, but instead of stimulating osteoblastic differentiation in MSCs, it has the opposite effect and inhibits the induction of osteoblastic differentiation of marrow stromal cells (MSCs); and it inhibits the activity of alkaline phosphatase, which is a marker of osteoblastic differentation. See, *e.g.*, Parhami *et al* (2002) *J. Bone & Mineral Res* 17, 1997, particularly the Abstract and page 1998, left column, second full paragraph. This published paper was filed in an IDS in the present application on December 12, 2008, and was considered by the Examiner. Another copy of the

paper is attached for the convenience of the Examiner. The Parhami *et al* (2002) reference, by showing that at least one type of statin fails to stimulate osteoblastic differentiation in MSCs, even though it inhibits HMG-CoA reductase, supports applicant's argument there is not a causal relationship between the ability of an agent to inhibit HMG-CoA reductase and its ability to stimulate osteoblastic differentiation in MSCs and, in fact, teaches away from there being any such causality. It was thus not at all predictable at the time of filing the application whether a particular agent that inhibits HMG-CoA reductase would stimulate or would inhibit osteoblastic differentiation of MSCs. These arguments, alone, are sufficient to counter the Examiner's allegation that Paralkar suggests or discloses that additional HMG-CoA reductase inhibitors, such as oxysterols, can be substituted for statins discussed in the reference, in order to achieve osteoblastic differentiation, with a reasonable expectation of success.

Furthermore, attached to this Reply is a Declaration under 37 CFR 1.132, executed by the inventor, attesting that at the time the application was filed, the mechanism by which agents can stimulate osteoblastic differentiation of MSCs was poorly understood. In particular, it was unpredictable whether agents that inhibit HMG-CoA reductase would stimulate or would inhibit osteoblastic differentiation of MSCs. Furthermore, the inventor refers in the Declaration to his contemporaneous studies which indicated that at least one statin, which inhibits HMG-CoA reductase, does *not* stimulate osteoblastic differentiation of MSCs, but rather inhibits osteoblastic differentiation. The inventor points out that his research group subsequently confirmed that oxysterols stimulate osteoblastic differentiation by an entirely different mechanism than that of certain statins. Unlike these statins, oxysterols do not exert their osteogenic effect via the expression of BMP, but rather function via aspects of the hedgehog pathway.

The **"Parish"** reference does not remedy the defects of Paralkar.

To address the defect in Paralkar that it does not specifically teach osteoblastic differentiation with an *oxysterol*, the Examiner refers to the Parish paper for its disclosure that side-chain oxysterols are potent inhibitors of HMG-CoA reductase. Parish does not mention osteoblastic differentiation. Even if this paper did disclose that side-chain oxysterols are potent inhibitors of HMG-CoA reductase, the paper does not remedy the defect of Paralkar that it fails to show that an oxysterol (or, for that matter, any particular inhibitor of HMG-CoA reductase) would be expected to induce osteoblastic differentiation. Furthermore, Parish does not suggest or

disclose that an inhibitor of HMG-CoA reductase can inhibit adipocyte differentiation of mammalian mesenchymal stem cells (as is required by claim 1).

The **"Wang "** reference does not remedy the defects of Paralkar, or of Paralkar taken with Parish.

The Examiner cites the Wang *et al.* reference for its alleged disclosure that treatment with statins (in this case, lovastatin) inhibits adipocyte differentiation and induces osteoblastic differentiation of mouse MSCs, as indicated by markers such as alkaline phosphatase activity, osteocalcin mRNA and cAMP production.

Like the other references cited by the Examiner, Wang is directed to the effects of a statin, and does not suggest or disclose that an oxysterol could substitute for the statin to, *e.g.*, induce osteoblastic differentiation. For this reason alone, the reference does not remedy the defects of Paralkar, or of Paralkar taken with Parish, which fail to teach that an oxysterol (or, for that matter, any particular inhibitor of HMG-CoA reductase) would be expected to induce osteoblastic differentiation.

Furthermore, the Wang reference does not directly test the ability of a statin - lovastatin - to inhibit adipocyte differentiation and induce osteoblastic differentiation of MSCs. Rather, this paper is directed to the development of a treatment that can preserve bone mass and prevent osteonecrosis in animals that are treated with steroids. That is, the paper studies the ability of the statin to *reverse* the effects of steroids (osteonecrosis, or the death of bone and bone cells) on marrow osteoprogenitor cells. The cells or animals are first treated with steroids, which induce fat expression and inhibit osteoblastic gene expression (or, in the animals, cause bone death, or osteonecrosis). The cells or animals are then treated with the statin to determine if it can reverse those phenomena. Even if Wang showed that statins can reverse the osteonecrotic effects of steroids (death of bone and bone cells), the reference does not show that the statins can induce osteogenic differentiation. For example, if steroids cause osteonecrosis in animals by inducing the death of osteoblasts, then the statins could be reversing this effect by inhibiting osteoblast death, rather than by stimulating osteogenesis.

Therefore, Wang *et al.*, either alone or in combination with Parish, does not remedy the defect of Paralkar, that it fails to suggest or disclose that an oxysterol (or, for that matter, any

particular inhibitor of HMG-CoA reductase) would be expected to induce osteoblastic differentiation in an MSC.

In addition, none of the cited references, taken alone or in combination with other cited references, suggests or discloses that the particular oxysterols or combinations of oxysterols that are recited in the present claims (*e.g.*, claims 2-3, 7-8, 16-17, 20-21, or 25-26) can induce osteoblastic differentiation and inhibit adipocyte differentiation of MSCs.

For at least the preceding reasons, neither the cited references nor the art at the time the invention was made provide a motivation to combine the cited references to achieve the presently claimed invention, with a reasonable expectation of success, and thus do not render the present claims obvious. Applicant requests that the obviousness rejection be withdrawn.

Obviousness-type double patenting rejections

Applicant, without acquiescing to any rejection, respectfully requests that the two provisional double-patenting rejections be held in abeyance until the allowance of claims in the instant application. While in no way admitting that the present claims are obvious over the claims of the cited applications, upon allowance of the claims of the instant application, Applicant will consider filing terminal disclaimers.

In view of the preceding arguments, applicant believed that the present claims are in condition for allowance, which action is respectfully requested.

Should any additional fee be deemed due, please charge such fee to our Deposit Account No. 22-0261, reference our docket number 58086-241892, and notify the undersigned accordingly

Respectfully submitted,

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Nancy Axelrod, Ph.D.
Registration No. 44,014
VENABLE LLP
P.O. Box 34385
Washington, D.C. 20043-9998
Telephone: (202) 344-4000
Telefax: (202) 344-8300

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